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Effect of hGC-MSCs from human gastric cancer tissue on cell proliferation, invasion and epithelial-mesenchymal transition in tumor tissue of gastric cancer tumor-bearing mice

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ABSTRACT

Objective: To study the effect of hGC-MSCs from human gastric cancer tissue on cell proliferation, invasion and epithelial-mesenchymal transition in tumor tissue of gastric cancer tumor-bearing mice.

Methods: BABL/c nude mice were selected as experimental animals and gastric cancer tumor-bearing mice model were established by subcutaneous injection of gastric cancer cells, randomly divided into different intervention groups. hGC-MSCs group were given different amounts of gastric cancer cells for subcutaneous injection, PBS group was given equal volume of PBS for subcutaneous injection. Then tumor tissue volume were determined, tumor-bearing mice were killed and tumor tissues were collected, mRNA expression of proliferation, invasion, EMT-related molecules were determined.

Results: 4, 8, 12, 16, 20 d after intervention, tumor tissue volume of hGC-MSCs group were significantly higher than those of PBS group and the more the number of hGC-MSCs, the higher the tumor tissue volume; mRNA contents of Ki-67, PCNA, Bcl-2, MMP-2, MMP-7, MMP-9, MMP-14, N-cadherin, vimentin, Snail and Twist in tumor tissue of hGC-MSCs group were higher than those of PBS group, and mRNA contents of Bax, TIMP1, TIMP2 and E-cadherin were lower than those of PBS group.

Conclusion: hGC-MSCs from human gastric cancer tissue can promote the tumor growth in gastric cancer tumor-bearing mice, and the molecular mechanism includes promoting cell proliferation, invasion and epithelial-mesenchymal transition.

1. Introduction

Mesenchymal stem cells (MSCs) are the stem cells from mesoderm of early embryo development that have the features such as highly self-renewal ability, easily transferring into exogenous genes and oncolytic virus, and weak immunogenicity [1,2]. Studies in recent years have confirmed that stem cells have good affinity to solid tumors and participate in the occurrence and development of a variety of malignant tumors. MSCs in vitro isolation, culture and amplification are relatively easy, and they are the ideal carrier for the verification of malignant tumor pathogenesis and the targeted treatment of malignant tumors [3–5]. However, the relationship between MSCs and the occurrence and development of gastric cancer is not yet clear at present, and in the following study, hGC-MSCs from human gastric cancer tissue were isolated and cultured, and gastric cancer tumor-bearing mice model were established, aiming at verifying the effect of hGC-MSCs from human gastric cancer tissue on tumor growth of gastric cancer tumor-bearing mice through animal experiments.

2. Materials and methods

2.1. Materials

Human gastric cancer cell lines SGC-7901 were bought from the cell bank of Chinese academy of sciences, BABL/c nude mice were purchased by the university animal center [license: SCXK (Su) 2011-0003], 1640 culture medium, fetal bovine serum and trypsin for cell culture were bought from Gibco Company, and the RNA extraction and PCR detection kits were bought from Beijing Tiangen Biotech Company.

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2.2. Tumor-bearing mouse model establishment

SGC-7901 cell lines were recovered, then cultured with 1640 culture medium containing 10% fetal bovine serum, amplified and then digested with trypsin, cell density was adjusted to 5×10^7/mL, 200 μL cell suspension was collected and subcutaneously inoculated in the back of BABL/c nude mice, the volume of tumor tissue was measured after 8 d, and those with volume more than 0.1 cm^3 were collected for subsequent research.

2.3. Separate culture and injection of hGC-MSCs

Fresh gastric carcinoma tissue was collected, saved under aseptic conditions, sent to the super clean bench, cut up to tissue fragments of 1 mm^3, then inoculated in a Petri dish for 2 h of adherence, culture medium was added for continuous incubation for 24 h, half the medium was changed, un-adhered tissue mass and cells were discarded, medium was changed every 3 d, cells were digested and sub-cultured after 80%–90% of fusion, and hGC-MSCs were obtained for treatment. HGC-MSCs group received injection of 0.5×10^6, 1.0×10^6 and 2.0×10^6 hGC-MSCs respectively around the transplantation tumor, and PBS group received injection the same volume of PBS.

2.4. Determination of tumor tissue growth

(1) 0, 4, 8, 12, 16 and 20 d after establishment of tumor-bearing mice model, the long diameter and short diameter of tumor were determined, and tumor tissue volume was calculated according to long diameter × short diameter × short diameter/2; (2) on the 21 d, tumor-bearing mice were executed and anatomized to get tumor tissue, RNA extraction kit was used to extract RNA and reverse-transcribe it into cDNA, PCR kit was used for amplification and detection, and mRNA contents of Ki-67, PCNA, Bcl-2, Bax, P53, MMP-2, MMP-7, MMP-9, MMP-14, TIMP1, TIMP2, E-cadherin, N-cadherin, Vimentin, Snail and Twist were calculated.

2.5. Statistical methods

SPSS20.0 software was used for analysis, differences among groups were compared by single factor analysis of variance, the pair-wise comparison was by LSD method and $P < 0.05$ indicated statistical significance in differences.

3. Results

3.1. hGC-MSCs promoted tumor tissue growth in tumor-bearing mice

Different doses of hGC-MSCs could all promote the tumor tissue growth in tumor-bearing mice. At each point in time, tumor tissue volume of hGC-MSCs group were significantly higher than those of PBS group, and the larger the dose of hGC-MSCs, the more significant the increase of tumor tissue volume, and it was significantly dose-dependent (see Table 1).

3.2. hGC-MSCs regulated the expression of Ki-67, PCNA, Bcl-2 and Bax in tumor tissue

Different doses of hGC-MSCs could all increase the expression of Ki-67, PCNA and Bcl-2, and inhibit the expression of Bax in tumor tissue. mRNA contents of Ki-67, PCNA and Bcl-2 in tumor tissue of hGC-MSCs group were higher than those of PBS group, and mRNA content of Bax was lower than that of PBS group; the larger the dose of hGC-MSCs, the more significant the change in the mRNA contents of Ki-67, PCNA, Bcl-2 and Bax, and it was significantly dose-dependent (see Table 2).

3.3. hGC-MSCs regulated the expression of MMPs and TIMPs in tumor tissue

Different doses of hGC-MSCs could all increase the expression of MMP-2, MMP-7, MMP-9 and MMP-14, and inhibit the expression of TIMP1 and TIMP2 in tumor tissue. mRNA contents of MMP-2, MMP-7, MMP-9 and MMP-14 in tumor tissue of hGC-MSCs group were higher than those of PBS group, and mRNA contents of TIMP1 and TIMP2 were lower than those of PBS group; the larger the dose of hGC-MSCs, the more significant the change in the mRNA contents of MMP-2, MMP-7, MMP-9, MMP-14, TIMP1 and TIMP2, and it was significantly dose-dependent (see Table 3).

3.4. hGC-MSCs regulated the expression of EMT-related molecules in tumor tissue

Different doses of hGC-MSCs could all increase the expression of N-cadherin, Vimentin, Snail and Twist, and inhibit the expression of E-cadherin in tumor tissue. mRNA contents of

Table 2
mRNA contents of proliferation-related molecules in tumor tissue of every group.

Groups	Proliferation-related molecules			
	Ki-67/β-actin	PCNA/β-actin	Bcl-2/β-actin	Bax/β-actin
2×10^6 hGC-MSCs	2.95 ± 0.32	3.38 ± 0.39	3.14 ± 0.33	0.29 ± 0.28
1×10^6 hGC-MSCs	2.11 ± 0.24	2.28 ± 0.24	2.39 ± 0.25	0.55 ± 0.07
0.5×10^6 hGC-MSCs	1.67 ± 0.18	1.74 ± 0.21	1.49 ± 0.15	0.78 ± 0.09
PBS group	1.00 ± 0.12	1.00 ± 0.11	1.00 ± 0.08	1.00 ± 0.14

Table 1
Tumor tissue volume of all groups (cm^3).

Groups	Action time					
	0 d	4 d	8 d	12 d	16 d	20 d
2×10^6 hGC-MSCs	0.105 ± 0.012	0.528 ± 0.065	1.229 ± 0.128	1.912 ± 0.228	2.585 ± 0.278	3.255 ± 0.339
1×10^6 hGC-MSCs	0.112 ± 0.011	0.394 ± 0.052	0.924 ± 0.104	1.449 ± 0.179	2.003 ± 0.231	2.783 ± 0.315
0.5×10^6 hGC-MSCs	0.101 ± 0.010	0.304 ± 0.036	0.683 ± 0.080	1.214 ± 0.097	1.583 ± 0.178	2.218 ± 0.252
PBS group	0.108 ± 0.009	0.194 ± 0.020	0.489 ± 0.057	0.881 ± 0.094	1.133 ± 0.128	1.331 ± 0.148

Table 3
mRNA contents of MMPs and TIMPs in tumor tissue of every group.

Groups	MMPs				TIMPs	
	MMP-2	MMP-7	MMP-9	MMP-14	TIMP1	TIMP2
2×10^6 hGC-MSCs	2.87 ± 0.31	3.22 ± 0.35	2.37 ± 0.28	2.75 ± 0.29	0.33 ± 0.05	0.21 ± 0.03
1×10^6 hGC-MSCs	2.10 ± 0.22	2.29 ± 0.25	1.89 ± 0.22	2.02 ± 0.22	0.57 ± 0.07	0.50 ± 0.06
0.5×10^6 hGC-MSCs	1.67 ± 0.19	1.75 ± 0.14	1.38 ± 0.16	1.69 ± 0.18	0.81 ± 0.09	0.76 ± 0.08
PBS group	1.00 ± 0.14	1.00 ± 0.08	1.00 ± 0.13	1.00 ± 0.12	1.00 ± 0.08	1.00 ± 0.17

N-cadherin, Vimentin, Snail and Twist in tumor tissue of hGC-MSCs group were higher than those of PBS group, and mRNA content of E-cadherin was lower than that of PBS group; the larger the dose of hGC-MSCs, the more significant the change in the mRNA contents of E-cadherin, N-cadherin, Vimentin, Snail and Twist, and it was significantly dose-dependent (see Table 4).

Table 4
mRNA contents of EMT-related molecules in tumor tissue of every group.

Groups	EMT marker molecules			EMT transcription factors	
	E-cadherin	N-cadherin	Vimentin	Snail	Twist
2×10^6 hGC-MSCs	0.28 ± 0.03	2.38 ± 0.29	2.84 ± 0.33	3.52 ± 0.39	3.24 ± 0.36
1×10^6 hGC-MSCs	0.49 ± 0.06	1.87 ± 0.21	2.04 ± 0.22	2.38 ± 0.25	2.13 ± 0.22
0.5×10^6 hGC-MSCs	0.72 ± 0.09	1.38 ± 0.15	1.54 ± 0.17	1.79 ± 0.22	1.59 ± 0.18
PBS group	1.00 ± 0.12	1.00 ± 0.08	1.00 ± 0.18	1.00 ± 0.14	1.00 ± 0.15

4. Discussion

Mesenchymal stem cells (MSCs) are a kind of adult stem cells with features such as highly self-renewal ability, easily transferring into exogenous genes and oncolytic virus, and weak immunogenicity, and are associated with the pathogenesis of a variety of malignant solid tumors. Studies believe that gastric cancer stem cells are derived from stem cells in the gastrointestinal tract, and the above stem cells are in the undifferentiated state from early embryo development, or dedifferentiated from mature embryos [6,7]; other studies have confirmed that gastric cancer stem cells are from bone marrow mesenchymal stem cell (BMSC), and BMSC can switch phenotype and become cancer stem cells causing gastric cancer [8,9]. Mesenchymal stem cells have good affinity to tumors, and can inter-play and interact with tumor microenvironment after affinity to tumor tissue, which cannot only adjust the biological characteristics of tumor cells, but can also change the biological characteristics of their own [10–13].

The relationship between MSCs and the occurrence and development of gastric cancer has received more and more attention and recognition, but most of the studies about MSCs and gastric cancer use MSCs from normal bone marrow, and cannot fully simulate the biological characteristics of MSCs in tumor microenvironment. Study has confirmed that compared with bone mesenchymal stem cells (BMSCs) from normal bone marrow, the expression of a variety of genes in mesenchymal stem cells (hGC-MSCs) from human gastric cancer tissue changes, and proliferation, invasion and other biological characteristics are also different [14]. In the study, hGC-MSCs were isolated from fresh human gastric cancer tissue and cultured, hGC-MSCs were injected around the tumor after establishment of gastric cancer tumor-bearing mouse model, and measuring the volume of tumors showed that hGC-MSCs could promote the growth of gastric cancer tissue in dose-dependent manner.

In the process of gastric cancer tissue growth, tumor cell proliferation, invasion and other biological behaviors are significantly enhanced, and the expression of a variety of proliferation and invasion-related molecules in tumor tissue is abnormal. HGC-MSCs can regulate the biological behavior of tumor cells, and in order to determine whether hGC-MSCs regulated the expression of proliferation and invasion-related molecules so as to affect the biological behavior of tumor cells, the contents of biological characteristics-related molecules in tumor proliferation were determined. PCNA is the accessory protein for DNA polymerase to be functioning, is the molecule necessary for intracellular DNA replication and cell proliferation, Ki-67 is the molecule closely related to the function of chromatin, and PCNA and Ki-67 are used as cell proliferation markers [15,16]. Bcl-2 and Bax are the key molecules regulating mitochondrial apoptosis, Bcl-2 can adjust the opening and closing of the mitochondrial membrane transition pore, reduce the release of cytochrome C and restrain the activation of apoptotic pathways, and Bax can form heterodimer with Bcl-2 to antagonize the antiapoptotic effect of Bcl-2 and promote cell apoptosis [17,18]. Analysis in the study showed that different doses of hGC-MSCs could all increase the expression of Ki-67, PCNA and Bcl-2, and inhibit the expression of Bax and P53 in tumor tissue.

Cancer cell invasion is a key link causing tumor tissue growth and distant metastasis, and can degrade and destruct the structural barrier formed by extracellular matrix and vascular wall basement membrane, invade the microcirculation and migrate to the distant. Matrix metalloproteinases (MMPs) play a major role in cell degradation of extracellular matrix [19]. MMP-2, MMP-7, MMP-9 and MMP-14 are the important members of MMPs family, and can degrade the composition such as collagen and laminin in the extracellular matrix. In addition, MMP-14 can also activate MMP-2, MMP-9 and various other protease molecules [20]. Tissue inhibitor of metalloproteases

(TIMPs) are the inhibitors of MMPs, and TIMP1 and TIMP2 can form covalent bonding with a variety of MMPs molecules to suppress their proteolysis function. Studies have confirmed that high expression of MMPs and lower expression of TIMPs are the important parts causing the occurrence and development of gastric cancer [21,22]. The analysis in the study showed that different doses of hGC-MSCs could increase the expression of MMP-2, MMP-7, MMP-9 and MMP-14, and inhibit the expression of TIMP1 and TIMP2 in tumor tissue.

Epithelial-mesenchymal transition (EMT) is an important pathological feature in the occurrence and development of gastric cancer cells, specifically refers to the phenomenon of epithelial cell differentiation to mesenchymal cells under certain conditions, and is specifically manifested that the epithelial cells lose polarity, cells get strong movement ability and intercellular adhesion ability is decreased [23,24]. E-cadherin is epithelial cell marker that can maintain cell polarity and adhesion; N-cadherin and Vimentin are mesenchymal markers that can enhance cell migration and invasion, and cause invasive cell growth [25]. In the process of EMT, epithelial cells transit to mesenchymal cells, the expression of E-cadherin is decreased significantly and the expression of N-cadherin and Vimentin is increased significantly. Snail and Twist are two transcription factors regulating cell EMT process, and can be combined with the DNA sequences of epithelial cell and mesenchymal cell markers to adjust their transcription and thus promote cell EMT [26,27]. In the study, analysis of EMT in gastric cancer tissue of tumor-bearing mice showed that different doses of hGC-MSCs could all promote the process of EMT in gastric cancer tissue, increase the expression of N-cadherin, Vimentin, Snail and Twist, and inhibit the expression of E-cadherin.

To sum up, hGC-MSCs from human gastric cancer tissue can promote the tumor growth in gastric cancer tumor-bearing mice, and the molecular mechanism includes promoting cell proliferation, invasion and epithelial-mesenchymal transition.

Conflict of interest statement

We declare that we have no conflict on interest.

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